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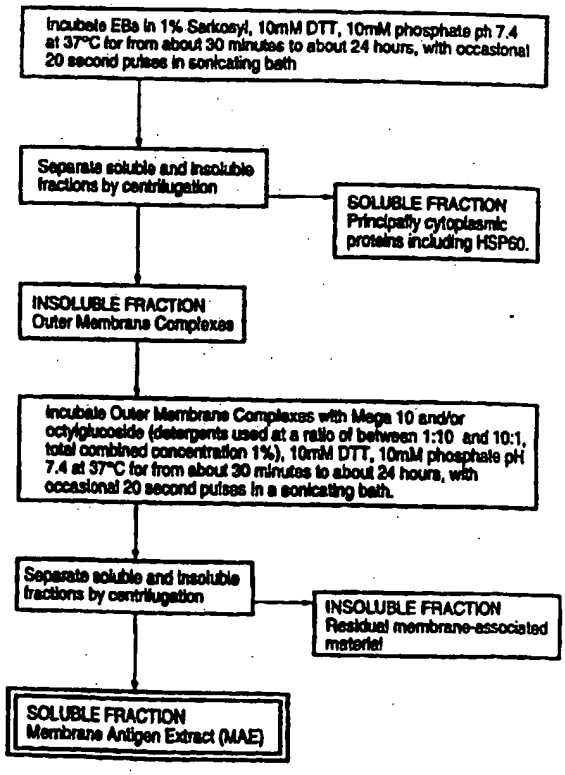
(54) Title: CHLAMYDIAL VACCINES AND IMMUNOGENIC COMPOSITIONS CONTAINING AN OUTER MEMBRANE ANTIGEN AND METHODS OF PREPARATION THEREOF

(57) Abstract

Immunogenic compositions including vaccines are described that comprise an outer membrane antigen extract of a strain of *Chlamydia* and are effective in protection against disease caused by *Chlamydia* infection. The immunogenic compositions may comprise the major outer membrane protein (MOMP) of *Chlamydia* which may be in a homooligomeric form or complexed with at least one other antigen of *Chlamydia*. The immunogenic composition may include an immunostimulating complex (ISCOM) and the outer membrane antigen may be incorporated therein. The immunogenic compositions have utility as chlamydial vaccines and in diagnostic applications.



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TITLE OF INVENTION

CHLAMYDIAL VACCINES AND IMMUNOGENIC COMPOSITIONS CONTAINING AN OUTER MEMBRANE ANTIGEN AND METHODS OF PREPARATION THEREOF

FIELD OF INVENTION

The invention relates to the field of immunology and, in particular, relates to vaccines against *Chlamydia*.

REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending United States Patent Application No. 08/713,236 filed September 12, 1996.

BACKGROUND TO THE INVENTION

Chlamydia trachomatis is a species of the genus Chlamydiaceae, order Chlamydiales. *C. trachomatis* infects the epithelia of the conjunctivae and the genital tract, causing trachoma and a variety of sexually transmitted diseases (STDs) which can lead to, respectively, blindness or infertility. There are at least 15 serovars of *C. trachomatis*, of which A, B, and C are causative agents of trachoma, while serovars D, E, F, G, H, I, J, and K are the most common causative agents of chlamydial STDs. *C. trachomatis* infections are endemic throughout the world. Trachoma is the leading cause of preventable blindness in developing nations, and it is estimated that 600 million people suffer from trachoma worldwide, with as many as 10 million of them being blinded by the disease. In the United States there are an estimated 3 million cases per year of STDs caused by *C. trachomatis*.

The pathogenesis of trachoma involves repeated ocular infections and the generation of a deleterious hypersensitivity response to chlamydial antigen(s) (refs 1 to 4 - Throughout this specification, various references are referred to in parenthesis to more fully

describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately following the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). The available evidence supports the hypothesis that both secretory IgA and cell-mediated immune responses are important components of protection. Ocular infection in a primate model induces rapid and persistent production of IgA in tears, whereas the presence of IgG in tears is transient, corresponding to the period of peak conjunctival inflammation (ref. 5). Protective immunity following experimental ocular infection in a sub-human primate model is homotypic and resistance to ocular challenge correlates with the presence of serovar-specific antibodies in tears (refs. 1, 2, 6). Tears from infected humans neutralised the infectivity of homologous but not heterologous trachoma serovars for owl monkey eyes (ref. 7) whereas passive humoral immunization with antitrachoma antibodies was not protective (ref. 8). Several lines of evidence indicate the importance of cell-mediated responses in protection from or clearance of chlamydial infection. B-cell deficient mice can resolve infection, whereas nude mice become persistently infected. Adoptive transfer of at least some chlamydia-specific T-cell lines or clones can cure persistently infected nude mice, and this anti-chlamydial activity is probably a function of the ability of the T-cells to secrete interferon-γ (refs. 9 to 17).

Past attempts to develop whole-cell vaccines against trachoma have actually potentiated disease by sensitizing vaccinees (refs. 1, 2). Sensitization has been determined to be elicited by a 57kD stress response protein (SRP) (HSP60) present in all serovars of *C. trachomatis*. Repeated exposure to the 57kD SRP can result in a delayed hypersensitivity reaction, causing

the chronic inflammation commonly associated with chlamydial infections. Thus, an immunogenic preparation capable of inducing a strong and enduring mucosal neutralising antibody response and a strong cellular immune response without sensitizing the vaccinee would be useful (ref. 18).

A most promising candidate antigen for the development of a vaccine is the chlamydial major outer membrane protein (MOMP) (refs. 19 to 21). Other surface proteins and the surface lipopolysaccharide are also immunogenic, but the antibodies they induce have not been found to be protective (ref. 22, 23). The MOMP, which is the predominant surface protein, is an integral membrane protein with a mass of about 40 kDa which, with the exception of four variable domains (VDs) designated I, II, III, and IV, is highly conserved amongst serovars. The sequences of all four VDs have been determined for fifteen serovars (ref. 24, 25). Antibodies capable of neutralising chlamydial infectivity recognize the MOMP (ref. 26, 27, 28, 29). Epitopes to which MOMP-specific neutralising monoclonal antibodies bind have been mapped for several serovars (refs. 22, 23, 30, 31, 32, 33, 34), and represent important targets for the development of synthetic or subunit vaccines. The binding sites are contiguous sequences of six to eight amino acids located within VDs I or II, and IV, depending on the serovar. Subunit immunogens (e.g. isolated MOMP or synthetic peptides) containing MOMP epitopes can induce antibodies capable of recognising intact chlamydiae (ref. 26). However, conventionally administered subunit immunogens are generally poor inducers of mucosal immunity. It would be useful to formulate chlamydial antigens in such a way as to enhance their immunogenicity and to elicit both humoral and cell-mediated immune responses.

Immune stimulating complexes (ISCOMs) are cage-like structures formed from a mixture of saponins (or saponin

derivatives), cholesterol and unsaturated fatty acids. The components of ISCOMs are held together by hydrophobic interactions, and consequently proteins which are naturally hydrophobic (such as MOMP) or which have been treated to expose or add hydrophobic residues can be efficiently incorporated into the ISCOMs as they form (ref. 35, 36, 37).

C. trachomatis naturally infects the mucosal surfaces of the eye and genital tract, and secretory IgA cellular responses are probably important components of protection. Consequently, it would be useful for a chlamydial vaccine to induce a mucosal immune response including both cellular and antibody components.

C. trachomatis infection may lead to serious disease. It would be advantageous to provide outer membrane antigen extracts of *Chlamydia*, including the major outer membrane protein of *Chlamydia*, particularly in substantially the native conformation for antigens in immunogenic preparations including vaccines, and immunogens and the generation of diagnostic reagents.

SUMMARY OF THE INVENTION

The present invention provides a novel immunogenic form of chlamydial MOMP which is useful in providing protection against chlamydial diseases, as well as methods of preparing such materials.

In accordance with one aspect of the invention, there is provided an immunogenic composition, comprising an outer membrane antigen extract (MAE) of a strain of *Chlamydia*, which may be *Chlamydia trachomatis*, and an immunostimulating complex (ISCOM).

The MAE may comprise the major outer membrane protein (MOMP) of the strain of *Chlamydia*. The MOMP may be in an oligomeric form and/or may be complexed with at least one other antigen of the strain of *Chlamydia*. Such

oligomers and complexes may have a molecular weight of from about 45 to about 125 kDa. The procedure described herein for preparation of the MAE, specifically MOMP, produces material which is substantially free from the heat shock protein HSP60 of the strain of *Chlamydia*. The immunogenic composition provided herein may be in the form of the MEA incorporated into ISCOMs.

The immunogenic compositions provided herein may be employed, in accordance with another aspect of the invention, to protect a host against disease caused by a strain of *Chlamydia* by administering to the host an effective amount of the immunogenic composition. Such administration may be to a mucosal surface to produce a mucosal immune response. Alternatively, any other convenient means of administration may be employed to produce the desired immune response. The administration may be to the mucosal surface of the host by intranasal administration and may produce a genital tract immune response. In addition, the immunogenic composition provided herein may be employed as a booster immunization in a prime-boost immunization procedure in which the prime immunization is effected with same form of *Chlamydia*, such as attenuated strain or a vector, including viral and bacterial vectors, containing a chlaymdial gene-expressing a chlamydial protein.

The present invention further includes a method of producing an outer membrane antigen extract of a strain of *Chlamydia* using a combination of two or more detergents including non-ionic detergents. Accordingly, in a further aspect of the invention, there is provided such a method which comprises:

detergent extracting elementary bodies (EBs) of the strain of *Chlamydia* in the presence of a reducing agent for disulphide bonds to solubilize cytoplasmic material away from outer membrane material;

separating the solubilized cytoplasmic material from the outer membrane materials,

detergent extracting the outer membrane material using at least two non-ionic detergents in the presence of a reducing agent for disulphide bonds to solubilize outer membrane antigens; and

separating the solubilized outer membrane antigens from residual unextracted membrane-associated material to provide the outer membrane antigen extract.

In one embodiment of this aspect of the invention, the at least two non-ionic detergents comprise a N-methyl glucamide non-ionic detergent which may be selected from heptanoyl-, octanoyl-, nonanoyl- and decanoyl-N-methyl glucamide, and a glucopyranoside non-ionic detergent, which may be selected from n-hexyl-β-D-, n-heptyl-β-D-, n-octyl-α-D-, n-octyl-β-D-, n-nonyl-β-D-, n-decyl-α-D- and n-decyl-β-D-glucopyranoside. Such glucopyranosides are sometimes termed glucosides. The two non-ionic detergents may be employed in a weight ratio from about 1:10 to about 10:1. The use of the two detergents enables a high degree of recovery of outer membrane antigen which remain soluble at a wide range of temperature of storage. Alternatively, the two detergents may be replaced by sodium dodecyl sulphate.

The procedure described herein for the preparation of the outer membrane extract produces a novel Chlamydial antigen preparation. Accordingly, in an additional aspect of the present invention, there is provided a purified outer membrane antigen extract of a strain of *Chlamydia* in the form of an aqueous solution of antigen in the presence of at least two non-ionic detergents and a reducing agent.

In such composition, the purified outer membrane antigen extract comprises the major outer membrane protein (MOMP) of the strain of *Chlamydia*, particularly

substantially in its native conformation. The MOMP usually comprises homooligomers and heterooligomers thereof, which may have the molecular weights from about 45 to about 125 kDa.

The provision of such novel purified materials enables there to be provided, in accordance with an additional aspect of the invention, a vaccine composition effective for protection against disease caused by a strain of *Chlamydia*, including *Chlamydia trachomatis*, comprising purified and non-denatured major outer membrane protein (MOMP) of the strain of *Chlamydia* substantially in its native conformation.

Such MOMP may be in the form of unaggregated homooligomers and heterooligomers. The vaccine composition may be in the form of immunostimulatory complexes (ISCOMs) incorporating the MOMP. The vaccine composition may further comprise at least one other chlamydial or non-chlamydial antigen.

The present invention further extends, in a further aspect of the invention, to a method for producing a vaccine against disease caused by a strain of *Chlamydia*, including *Chlamydia trachomatis*, comprising:

administering the vaccine composition provided herein to a test host to determine an amount and a frequency of administration thereof to confer protection against disease caused by the strain of *Chlamydia*; and

formulating the vaccine in a form suitable for administration to a treated host, which may be a human, in accordance with the determined amount and frequency of administration.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be further understood from the following description with reference to the accompanying drawings, in which:

Figure 1 shows the procedure for the purification of the chlamydial membrane antigen extract (MAE), in accordance with one aspect of the invention;

Figure 2, comprising three panels, are SDS-PAGE gels showing the results of preparing the MAE using octyl glucoside (upper panel), Mega 10 (middle panel), or a mixture of the two (lower panel) to extract the outer membrane complexes in the purification scheme of Figure 1. MW = molecular weight markers (kDa); EB = elementary bodies; Lane 1 = soluble fraction obtained after Sarkosyl extraction; Lane 2 = membrane antigen extract; Lane 3 = insoluble fraction containing residual membrane associated material;

Figure 3 shows the results of an immunoblot demonstrating the presence of HSP60 in the Sarkosyl-soluble fraction (lane 1) but not in the MAE (lane 3). Figure 3 also shows the presence in the MAE of MOMP (lanes 2 and 5) and several other antigens (lanes 2 and 4);

Figure 4 shows the results of an immunoblot conducted under reducing and non-reducing conditions demonstrating that MOMP in the MAE is present as oligomers (lane NR) with a molecular weights greater than that of monomeric MOMP (lane R). The indicated molecular weights (kDa) are actual molecular weights for the materials;

Figure 5, comprising panels A and B, are photomicrographs of ISCOMs prepared using the methods of Morein (ref. 36) (A) or of Mowat and Reid (ref. 37) (B), as described in the Examples below, and provided in accordance with an aspect of the present invention;

Figure 6 contains bar graphs showing the immunogenicity of ISCOMs containing MAE administered to mice intra-nasally or intra-peritoneally, in comparison to Freund's adjuvanted MAE administered intra-

peritoneally. ISCOMs administered by either route elicit both IgG and IgA in vaginal secretions;

Figure 7 contains graphical representations of the response of uninfected C3H mice to MAE-ISCOMs for different manners of preparation of the MAE-ISCOMs and different routes of inoculation. Log_{10} serum IgG anti-MOMP titres are shown at different stages of inoculation; and

Figure 8 contains graphical representations of the response of previously infected C3H mice to MAE-ISCOMs for different manners of preparation of the MAE-ISCOMS and different routes of inoculation. Log_{10} serum IgG anti-MOMP titres are shown at different stages of inoculation.

GENERAL DESCRIPTION OF INVENTION

The present invention provides novel techniques which can be employed for preparing outer membrane antigen extracts from *Chlamydia*, including purified major outer membrane protein from *Chlamydia*. Any *Chlamydia* strain, including *C. trachomatis*, may be conveniently used to provide the outer membrane antigen extracts as provided herein. Such strains are generally available from clinical sources and from bacterial culture collections.

Referring to Figure 1, there is illustrated, in the form of a flow chart, a procedure for the purification of the chlamydial outer membrane antigen extract (MAE). Thus, purified elementary bodies (EBs) are resuspended in 10mM phosphate buffer, pH 7.4, and made to 1 wt% Sarkosyl, 10mM dithiothreitol (DTT). The mixture is incubated at 37°C for from about 30 minutes to about 24 hours, with occasional 20-second pulses in a sonicating water bath. Following the incubation, soluble and insoluble fractions are separated by centrifugation at 150,000g for 1 hour at 20°C. The insoluble fraction comprises outer membrane complexes, which are recovered

as a pellet, while soluble material remains in the supernatant. The insoluble fraction is resuspended in 10mM phosphate buffer, pH 7.4, containing 10mM DTT, and decanoyl-N-methylglucamide (Mega 10) and/or octyl glucoside at a total combined concentration of about 1 wt%. The resuspended material is incubated at 37°C for from about 30 minutes to about 24 hours, with occasional 20-second pulses in a sonicating water bath. Following the incubation, soluble and insoluble fractions are separated by centrifugation at 150,000g for 1 hour at 20°C. The material remaining in the supernatant is the MAE.

Figure 2 illustrates the preparation of MAE using octyl glucoside, Mega 10, or a mixture of the two detergents, to extract the membrane antigens from the Sarkosyl-insoluble pellet. When the MAE is prepared using Mega 10 alone, or a mixture of octyl glucoside and Mega 10 in, for example, the ratio 1:3, the final insoluble pellet contains less MOMP than when only octyl glucoside is used. MW = molecular weight markers; EB = elementary bodies; Lane 1 = soluble fraction obtained after Sarkosyl extraction; Lane 2 = membrane antigen extract; Lane 3 = insoluble fraction containing residual membrane associated material.

Figure 3 illustrates the composition of the MAE as determined by immunoblotting. Using a rabbit antiserum specific for the cytoplasmic protein HSP60 shows that there is HSP60 in the Sarkosyl-soluble fraction (lane 1) but not in the MAE (lane 3). The major component of the MAE is the chlamydial major outer membrane protein (MOMP) as shown by immunoblotting with pooled strain-specific convalescent mouse antisera (lane 2) or with a MOMP-specific monoclonal antibody (lane 5). However, several other antigenic components of the MAE can be demonstrated using sera raised to the homologous strain of chlamydia (lane 2) or to a heterologous strain (lane 5).

Figure 4 shows the results of an immunoblot conducted under reducing and non-reducing conditions. These results demonstrate that MOMP in the MAE is present as oligomers (lane NR) with molecular weights ranging from 45 to 125 kDa. Monomeric MOMP of molecular weight about 39,000 Da is shown in Lane R.

Figure 5 illustrates ISCOMs containing MAE prepared using the methods of Morein (ref. 36) (A) or of Mowat and Reid (ref. 37) (B). When following the method of Morein, ISCOMs are prepared by diluting the MAE to about 0.2 mg/mL with 10mM phosphate buffer pH 6.8. Phosphatidyl choline and cholesterol are dissolved at about 5 mg/mL each in approximately 20% Mega 10 then added to the diluted MAE to a final concentration of about 0.2 mg/mL each. Quil A is added to a concentration of about 1 mg/mL. Sufficient 20% Mega 10 is then added to bring the final concentration in the mixture to about 1%. The mixture is incubated with shaking at room temperature overnight then dialysed at 20° to 25°C against three changes of 10mM phosphate buffer, pH 6.8, for from about 2 hours to about 20 hours per change. When prepared according to this method the ISCOMs are uniform particles about 40 to 50nm in diameter.

When following the method of Mowat and Reid, the membrane antigen extract is adjusted to a protein concentration of about 0.5 to 1 mg/mL and to a detergent concentration of about 2%. Quil A is added to a concentration of about 1 mg/mL. Phosphatidyl choline and cholesterol are dissolved at about 10mg each per mL in approximately 2% Mega 10 or octyl glucoside, then added to the membrane antigen extract at a concentration of about 0.5 mg each per mL. The mixture is mixed, then dialysed at 20° to 25°C against six changes of 50 mM Tris-HCl, pH 8.5 for about from about 6 hours to about 18 hours per change. When prepared according to this method

the ISCOMs vary in diameter from about 30nm to about 200nm.

Figure 6 shows the immunogenicity of ISCOMs containing MAE administered to mice either intranasally or intraperitoneally. Female A/J mice were inoculated with MAE-ISCOMs containing about 0.25 µg of protein by the intraperitoneal (mice #63-64) or the intranasal (mice #65-68) routes on days 1 and 14, or with MAE containing about 1 µg of protein in complete Freund's adjuvant on day 1 and with MAE containing about 1 µg of protein in incomplete Freund's adjuvant on day 14 (mice #9-12). Sera and vaginal washes were taken on days 0 and 28, and assayed in an ELISA assay for MAE-specific serum IgG, serum IgA, vaginal IgG and vaginal IgA. The MAE-ISCOMs induce serum IgG titres comparable to those induced by the higher dose of MAE in Freund's, and consistently induce vaginal IgG and IgA, which MAE in Freund's did not.

Figures 7 and 8 show the serum IgG anti-MOMP ELISA antibody responses obtained from innoculation of uninfected mice (Figure 7) and mice previously infected with *Chlamydia trachomatis* by intranasal and intramuscular innoculation using various MAE-ISCOM preparations. The immunization induced specific serum IgG responses in most uninfected animals immmunized with a vaccine containing MAE. Previously-infected animals had high pre-existing specific serum IgG responses which increased modestly following intramuscular immunization.

In the experiments performed, the intra-muscular route was more effective than the intranasal route at inducing serum IgG responses. While serum specific IgG responses were observed using the MAE-ISCOMs provided herein, animals immunized with ISCOM matrix alone did not produce a specific serum IgG response.

Histological studies were also carried out and inflammatory lesions in the uterus and oviducts of immunized mice were assessed as absent (normal tissue), mild or severe, according to the criteria outlined in Table 1 below. The results obtained are set forth in Table 2 below. As detailed below, certain groups of mice which had received MAE-ISCOMs were significantly protected from the development of lesions.

Advantages of the MAE-ISCOMs of the present invention include the capability to induce a strong and protective anti-chlamydial immune response when administered to a mammal without exacerbating chlamydial disease, by, for example, potentiation of chlamydial disease by sensitising vaccinees to HSP60.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of *Chlamydia* infections and the generation of immunological reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from immunogenic outer membrane antigen extracts of *Chlamydia*, including the major outer membrane protein (MOMP), as disclosed herein. The immunogenic composition elicits an immune response which produces antibodies, including anti-MOMP antibodies, IgG and IgA antibodies, and antibodies that are opsonizing or bactericidal. Should the vaccinated subject be challenged by *Chlamydia*, the antibodies bind thereto and thereby inactivate the *Chlamydia*. Furthermore, opsonizing or bactericidal antibodies may also provide protection by alternative mechanisms. The immunogenic compositions also produce cell mediated immune responses

including CD4+ and CD8+ T cell response including cytotoxic T cell responses specific for MOMP.

Immunogenic compositions including vaccines may be prepared as injectables, as liquid solutions or emulsions. The outer membrane antigen extracts may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously or intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient(s) in the range of about 0.5 to about 10%, preferably about 1 to 2%. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the outer membrane antigen extract, preferably about 20 to about 75%.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the outer membrane antigen extract per vaccination. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host.

The concentration of the outer membrane antigen extracts in an immunogenic composition according to the invention is in general about 1 to 95%. A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Thus, the immunogenic compositions provided herein may be formulated to comprise at least one additional immunogen, which may comprise or be derived from bacterial, viral or protozal pathogens including a paramyxovirus, polio, hepatitis B, bacterial toxoids, including diphtheria toxoid and tetanus toxoid, influenza, haemophilus, pertussis, pneumococcus,

mycobacteria, hepatitis A, HIV, HSV, *Neisseria gonorrhoea*, *Treponema pallidum*, and organisms which result in other sexually transmitted diseases.

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza

vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

2. Immunoassays

The outer membrane antigen extracts of the present invention are useful as immunogens for the generation of anti-*Chlamydia* antibodies and as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of anti-*Chlamydia* antibodies. In ELISA assays, the outer membrane antigen extract is immobilized onto a selected surface, for example, a surface capable of binding proteins such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antigen, a nonspecific protein such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex

(antigen/antibody) formation. This may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound outer membrane antigen extract, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second antibody may have an associated activity such as an enzymatic activity that will generate, for example, a colour development upon incubating with an appropriate chromogenic substrate. Quantification may then be achieved by measuring the degree of colour generation using, for example, a spectrophotometer.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1:

This Example illustrates the preparation of the membrane antigen extract from chlamydial elementary bodies, as shown in Figure 1.

Purified elementary bodies (EBs), prepared as described in ref. 19, were resuspended in 10mM phosphate buffer, pH 7.4, and made to 1 wt% Sarkosyl (N-Lauylsarosine, sodium salt), 10mM DTT. The EBs were incubated at 37°C for about 90 minutes, with occasional 20 second pulses in a sonicating water bath. Following the incubation, soluble and insoluble fractions were separated by centrifugation at 150,000g for 1 hour at 20°C. The insoluble fraction comprises outer membrane complexes which are recovered as a pellet, while soluble material comprising principally cytoplasmic proteins including HSP60, remains in the supernatant. The insoluble fraction was resuspended in 10mM phosphate buffer, pH 7.4, containing 10mM DTT, and decanoyl-N-methylglucamide (Mega 10) and/or octyl glucoside at a total combined concentration of about 1 wt%. The resuspended material was incubated at 37°C for about 90 minutes, with occasional 20-second pulses in a sonicating water bath. Following the incubation, soluble and insoluble fractions were separated by centrifugation at 150,000g for 1 hour at 20°C. The soluble material remaining in the supernatant was the membrane antigen extract while the insoluble fraction contained residual membrane-associated material.

Example 2:

This Example illustrates the preparation of ISCOMs with the membrane antigen extract according to the methods of Morein (ref. 36) or of Mowat and Reid (ref. 37).

When following the method of Morein, ISCOMs were prepared by diluting the MAE, prepared as described in Example 1, to about 0.2mg/ml with 10mM phosphate buffer pH 6.8. Phosphatidyl choline and cholesterol were dissolved at about 5mg/ml each in approximately 20% Mega 10 and then added to the diluted MAE to a final concentration of 0.2 mg/ml each. Quil A (a complex but purified mixture of *Quillaja* saponins which are glycosides of quillaic acid and carbohydrates) was added to a concentration of about 1 mg/ml. Sufficient 20% Mega 10 was then added to bring the final concentration in the mixture to about 1%wt. The mixture was shaken at room temperature overnight and then dialysed at 20 to 25°C against three changes of 10mM phosphate buffer, pH 6.8, for about 6 hours, about 16 hours and about 6 hours for the three buffer changes. When prepared according to this method, the ISCOMs were uniform particles about 40 to 50nm in diameter.

When following the method of Mowat and Reid, the membrane antigen extract, prepared as described in Example 1, was diluted to a protein concentration of about 0.5 to 1 mg/ml and to a detergent concentration of about 2 wt%. Quil was added to a concentration of about 1 mg/ml. Phosphatidyl choline and cholesterol were dissolved at about 10mg each per ml in approximately 2% Mega 10 or octyl glucoside, then added to the membrane antigen extract at a concentration of about 0.5 mg each per ml. The mixture was mixed briefly, then dialysed at 20 to 25°C against six changes of 50 mM Tris-HCl, pH 8.5 alternately for about 6 hours and about 18 hours per buffer change. When prepared according to this method,

the ISCOMs vary in diameter from about 30nm to about 200nm.

Electron micrographs of ISCOMs formed by both methods are shown in Figure 5. Panel A shows the ISCOMs prepared according to the procedure of Morein and Panel B shows the ISCOMs prepared according to the procedure of Mowat and Reid.

Example 3:

This Example illustrates the immunogenicity of chlamydial membrane antigen extract (MAE)-ISCOMs in mice.

Female A/J mice were immunized with MAE-ISCOMs, prepared as described in Example 2 following the procedure of Mowat and Reid, containing about 0.25 µg of protein by the intraperitoneal (mice #63-64) or the intranasal (mice #65-68) routes on days 1 and 14, or with MAE containing about 1 µg of protein in complete Freund's adjuvant on day 1 and with MAE containing about 1 µg of protein in incomplete Freund's adjuvant on day 14 (mice #9-12). Sera and vaginal washes were taken on days 0 and 28, and assayed in an ELISA assay for MAE-specific serum IgG, serum IgA, vaginal IgG and vaginal IgA.

As may be seen from the results obtained (Figure 6), the MAE-ISCOMs provided herein induced serum IgG titres comparable to those induced by the higher dose of MAE in Freund's (upper panels), and consistently induced vaginal IgG (penultimate panels). MAE in Freund's adjuvant did not induce any IgA antibodies whereas the MAE-ISCOMs produced IgA antibodies (lower panels).

Example 4:

This Example illustrates the use of MAE-ISCOMs to protect mice from chlamydial infection.

A group of 160 female mice C3H, aged 6 to 8 weeks, were divided into two groups of 80, designated infected and uninfected. On days 0 and 7, all mice were treated with 2.5mg progesterone administered subcutaneously. On

day 7, the infected group was vaginally inoculated with 1000 ID_{50} of *Chlamydia trachomatis* MoPn strain and the uninfected group was vaginally inoculated with SPG buffer. Inoculations were performed under light anaesthesia. The animals were then rested until day 91, when they were further divided into sixteen groups and vaccinated as follows:

10 infected mice received intramuscularly MAE-ISCOMs prepared by the procedure of Morein as described in Example 2,

10 infected mice received intranasally MAE-ISCOMs prepared by the procedure of Morein as described in Example 2,

10 infected mice received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, in which the MAE was prepared as described in Example 1 except that sodium dodecyl sulphate was used in place of Mega 10 and/or octyl glucoside, intramuscularly,

10 infected mice received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, in which the MAE was prepared as described in Example 1 except that sodium dodecyl sulphate was used in place of Mega 10 and/or octyl glucoside, intranasally,

10 infected mice received MAE, prepared as described in Example 1, mixed with ISCOM matrix, intramuscularly,

10 infected mice received MAE, prepared as described in Example 1, mixed with ISCOM matrix, intranasally,

10 infected mice received ISCOM matrix intramuscularly,

10 infected mice received ISCOM matrix intranasally,

10 uninfected mice received intramuscularly MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2,

10 uninfected mice received intranasally MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2,

10 uninfected mice received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, in which the MAE was prepared as described in Example 1 except that sodium dodecyl sulphate was used in place of Mega 10 and/or octyl glucoside, intramuscularly,

10 uninfected mice received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, in which the MAE was prepared as described in Example 1 except that sodium dodecyl sulphate was used in place of Mega 10 and/or octyl glucoside, intranasally,

10 uninfected mice received MAE, prepared as described in Example 1, mixed with ISCOM matrix, intramuscularly,

10 uninfected mice received MAE, prepared as described in Example 1, mixed with ISCOM matrix, intranasally,

10 uninfected mice received ISCOM matrix intramuscularly,

10 uninfected mice received ISCOM matrix intranasally.

The ISCOM matrix employed was prepared in the same way as the MAE-ISCOMs as in Example 2 except that MAE was omitted from the reaction mixture. MAE mixed with ISCOM matrix was prepared by adding MAE to preformed ISCOM matrix. Each dose of vaccine contained about 2 µg of MAE and about 10 µg of saponin. These vaccinations were repeated on about days 112 and 133. Blood and vaginal washes were taken just before each vaccination and assayed in an ELISA assay for antigen specific serum IgG. As may be seen from the results obtained (Figures 7, 8) the immunizations induced specific serum IgG responses in most uninfected animals immunized with a vaccine containing MAE. Previously infected animals had high pre-existing specific serum IgG responses as a consequence of the infection which increased modestly following intramuscullar immunization. The intra-

muscular route was more affective than the intranasal route at inducing specific serum IgG responses. MAE-ISCOMs prepared by the procedure of Morein as described in Example 2 were more effective at inducing specific serum IgG responses than MAE-ISCOMs in which the MAE was prepared as described in Example 1 except that sodium dodecyl sulphate was used in place of Mega 10 and/or octyl glucoside. Animals immunized with ISCOM matrix alone did not produce a specific serum IgG response.

All mice were challenged with about 100 ID_{50} of *Chlamydia trachomatis* MoPn strain, administered vaginally to anaesthetized animals on about days 145, 147 and 149. On about days 154 and 161 three mice from each of the 12 groups were necropsied. On day 168 all remaining mice were necropsied. At necropsy, the reproductive tract was removed and divided into parts so that symptoms due to infection were determined by examination of histological sections.

Inflammatory lesions in the uterus and oviducts were assessed as absent (normal tissue), mild or severe, according to the criteria in Table 1. As may be seen from the results shown in Table 2, the following groups of mice were significantly protected from the development of lesions:

uninfected mice which received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, intramuscularly;

infected mice which received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, intramuscularly;

infected mice which received MAE-ISCOMs, prepared by the procedure of Morein as described in Example 2, intranasally;

infected mice which received MAE-ISCOMs, in which the MAE was prepared as described in example 1 except

that sodium dodecyl sulphate was used in place of Mega 10 and/or octyl glucoside, intramuscularly; and

infected mice which received MAE prepared as described in Example 1 mixed with ISCOM matrix, intramuscularly.

The presence of chlamydiae in the tract can be assessed immunologically and by PCR assay.

SUMMARY OF INVENTION

In summary of this disclosure, the present invention provides ISCOM, chlamydial major outer membrane protein complexes, useful in vaccines against chlamydial diseases, and in the preparation of immunological reagents. Modifications are possible within the scope of the invention.

TABLE 1
LESION SEVERITY

LESION	DESCRIPTION
	UTERUS
Absent	Normal uterus. (May have occasional mild foci of inflammation)
Mild	Mild to moderate inflammation of the tissues but little or no infiltrate in the uterine lumen.
Severe	Moderate to severe tissue inflammation with widespread infiltration into the uterine lumen.
	OVIDUCTS
Absent	Normal oviduct.
Mild	Mild inflammation of oviduct walls and supporting tissues, may have a few leukocytes in the oviduct lumen. Tissue architecture essentially normal. **OR** Moderate and widespread inflammation of oviduct walls and supporting tissues. Usually some localised infiltration into the oviduct lumen, but little damage to lumenal epithelium.
Severe	Extensive infiltration into the oviduct lumen. Lumenal epithelium still present, but microvilli flattened or absent. **OR** Extensive infiltration into the oviduct lumen. Lumenal epithelium absent or severely damaged.

TABLE 2

(1) Uninfected C3H mice

ADJUVANT	ANTIGEN	ROUTE OF IMMUNIZATION					
		INTRANASAL			INTRAMUSCULAR		
		Absent	Lesions Mild	Severe	Absent	Lesions Mild	Severe
Formulated ISCOMs	MAE	4/9	5/9	0/9	8/9 *0.024	0/9	1/9
Formulated ISCOMs	MAE prepared using SDS	5/10	2/10	3/10	5/8	3/8	0/8
ISCOM Matrix	MAE	5/10	4/10	1/10	3/10	6/10	1/10
ISCOM Matrix	None	3/8	0/8	5/8	4/9	4/9	1/9

* = p values versus combined controls

2) Previously infected C3H mice

ADJUVANT	ANTIGEN	ROUTE OF IMMUNIZATION					
		INTRANASAL			INTRAMUSCULAR		
		Absent	Lesions Mild	Severe	Absent	Lesions Mild	Severe
Formulated ISCOMs	MAE	5/10 *0.009	5/10	0/10	3/7 *0.042	4/7	0/7
Formulated ISCOMs	MAE prepared using SDS	0/10	8/10	2/10	4/10 *0.031	6/10	0/10
ISCOM Matrix	MAE	1/8	7/8	0/8	5/9 *0.005	4/9	0/9
ISCOM Matrix	None	1/10	9/10	0/10	0/10	10/10	0/10

* = p values versus combined controls

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CLAIMS

What we claim is:

1. An immunogenic composition, comprising an outer membrane antigen extract (MAE) of a strain of *Chlamydia* and an immunostimulating complex (ISCOM).

2. The immunogenic composition of claim 1 wherein said MAE comprises the major outer membrane protein (MOMP) of the strain of *Chlamydia*.

3. The immunogenic composition of claim 2 wherein said MOMP is in an oligomeric form and/or is complexed with at least one other antigen of the strain of *Chlamydia*.

4. The immunogenic composition of claim 3 wherein said oligomer and/or complexes have a molecular weight of from about 45 to about 125 kDa.

5. The immunogenic composition of claim 2 wherein said MOMP is substantially free from the heat shock protein HSP60 of the strain of *Chlamydia*.

6. The immunogenic composition of claim 1, wherein the outer membrane antigen extract is incorporated into immunostimulatory complexes (ISCOMs).

7. A method of protecting a host against disease caused by a strain of *Chlamydia*, comprising administering to said host an effective amount of the immunogenic composition of claim 1.

8. The method of claim 7 wherein said administration is to a mucosal surface of said host to produce a mucosal immune response.

9. The method of claim 8 wherein said administration to said mucosal surface is by intranasal administration to produce a genital tract immune response.

10. A method of producing an outer membrane antigen extract of a strain of *Chlamydia*, which comprises:

detergent extracting elementary bodies of said strain of *Chlamydia* in the presence of a reducing agent

to solubilize cytoplasmic material away from outer membrane material;

separating said solubilized cytoplasmic material from the outer membrane materials;

detergent extracting said outer membrane material using at least two non-ionic detergents in the presence of a reducing agent to solubilize outer membrane antigens; and

separating said solubilized outer membrane antigens from residual unextracted membrane-associated material to provide said outer membrane antigen extract.

11. The method of claim 10 wherein said at least two non-ionic detergents comprise a N-methylglucamide non-ionic detergent and a glucopyranoside non-ionic detergent.

12. The method of claim 11 wherein said N-methylglucamide non-ionic detergent is selected from the group consisting of heptanoyl-, octanoyl-, nonanoyl- and decanoyl-N-methylglucamide.

13. The method of claim 12 wherein said glucopyranoside non-ionic detergent is selected from the group consisting of n-hexyl-β-D-, n-heptyl-β-D-, n-octyl-α-D-, n-octyl-β-D-, n-nonyl-β-D-, n-decyl-α-D- and n-decyl-β-D-glucopyranoside.

14. The method of claim 13 wherein said two non-ionic detergents are employed in a weight ratio from about 1:10 to about 10:1.

15. A purified outer membrane antigen extract of a strain of *Chlamydia* in the form of an aqueous solution of antigen in the presence of at least two non-ionic detergents and a reducing agent.

16. The composition of claim 15 wherein said purified outer membrane antigen extract comprises the major outer membrane protein (MOMP) of the strain of *Chlamydia*.

17. The composition of claim 16 wherein said major outer membrane protein is in substantially its native conformation.

18. The composition of claim 17 wherein said major outer membrane protein comprises homooligomers and heterooligomers thereof.

19. A vaccine composition effective for protection against disease caused by a strain of *Chlamydia*, comprising purified major outer membrane protein (MOMP) of the *Chlamydia* strain substantially in its native conformation.

20. The vaccine composition of claim 19 wherein said MOMP is in the form of unaggregated homooligomers and heterooligomers.

21. The vaccine composition of claim 20 in the form of immunostimulatory complexes (ISCOMs) incorporating the MOMP.

22. The vaccine composition of claim 19 further comprising at least one other antigen.

23. A method for producing a vaccine against disease caused by a strain of *Chlamydia*, comprising:

administering the vaccine composition of claim 19 to a test host to determine an amount and a frequency of administration thereof to confer protection against disease caused by the *Chlamydia* strain; and

formulating the vaccine in a form suitable for administration to a treated host in accordance with said determined amount and frequency of administration.

24. The method of claim 23 wherein the treated host is a human.

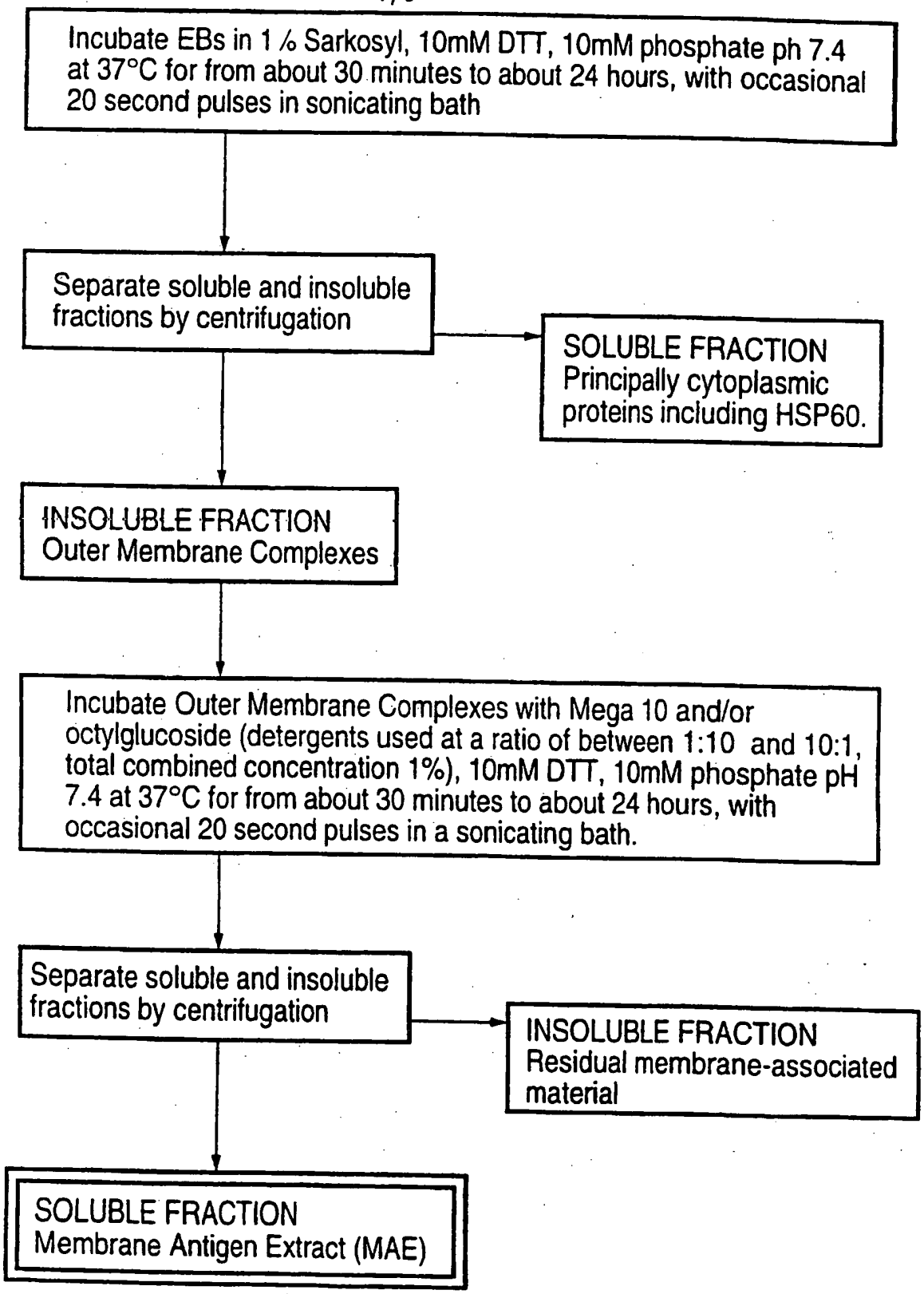

Incubate EBs in 1 % Sarkosyl, 10mM DTT, 10mM phosphate ph 7.4 at 37°C for from about 30 minutes to about 24 hours, with occasional 20 second pulses in sonicating bath
Separate soluble and insoluble fractions by centrifugation
SOLUBLE FRACTION Principally cytoplasmic proteins including HSP60.
INSOLUBLE FRACTION Outer Membrane Complexes
Incubate Outer Membrane Complexes with Mega 10 and/or octylglucoside (detergents used at a ratio of between 1:10 and 10:1, total combined concentration 1%), 10mM DTT, 10mM phosphate pH 7.4 at 37°C for from about 30 minutes to about 24 hours, with occasional 20 second pulses in a sonicating bath.
Separate soluble and insoluble fractions by centrifugation
INSOLUBLE FRACTION Residual membrane-associated material
SOLUBLE FRACTION Membrane Antigen Extract (MAE)

FIG.1

FIG. 2A.

Extraction using
1% Octyl glucoside

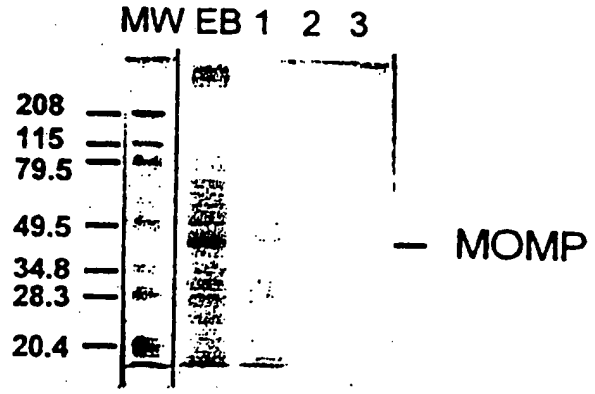


FIG. 2B.

Extraction using
1% Mega 10

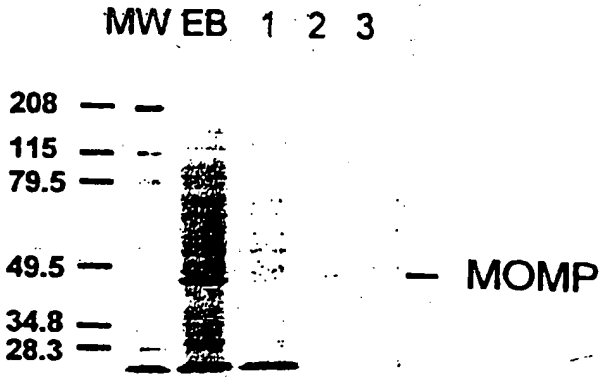


FIG. 2C.

Extraction using
0.75% Octyl glucoside
+ 0.25% Mega 10

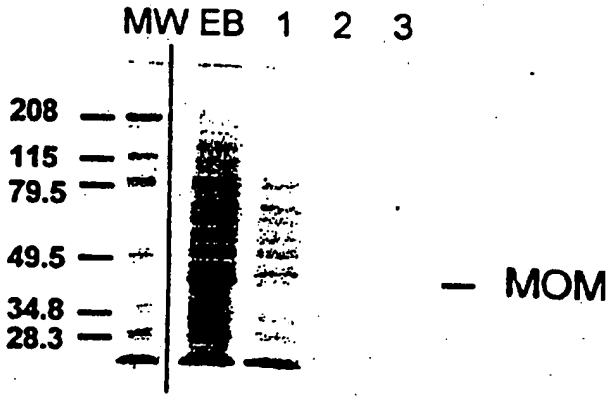
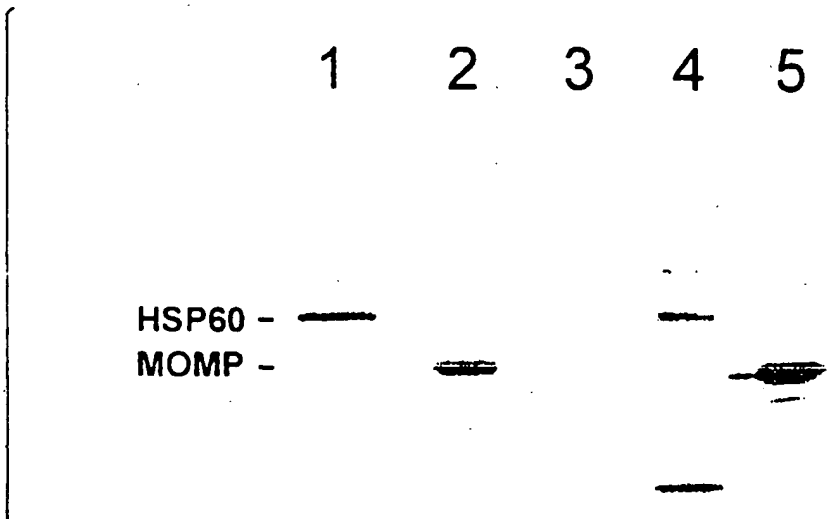
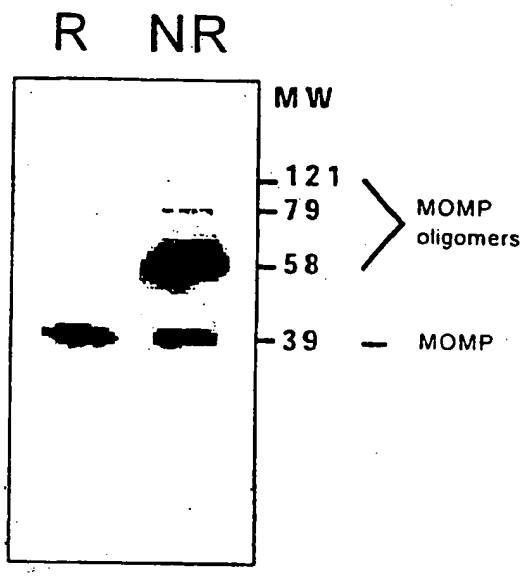


Figure 3.



1. Sarkosyl-soluble fraction probed with anti-HSP60
2. MAE probed with convalescent mouse serum
3. MAE probed with anti-HSP60
4. MAE probed with anti-serovar L2
5. MAE probed with mAb anti-MOMP

Figure 4.



R = MAE analysed by reducing SDS-PAGE
NR = MAE analysed by non-reducing SDS-PAGE
MW = Approximate molecular weight of MOMP and oligomers

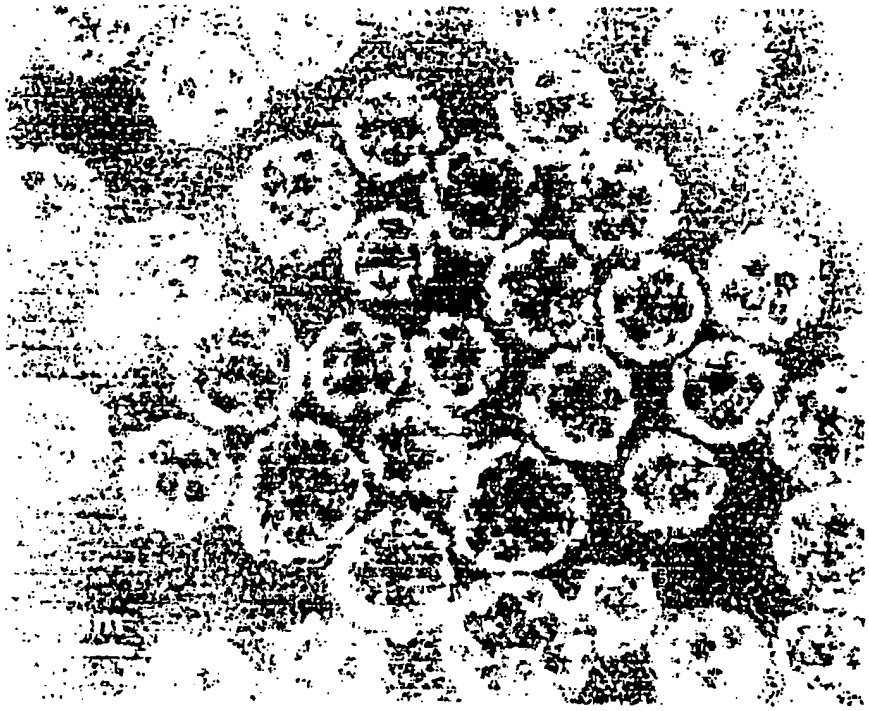


FIG 5A.

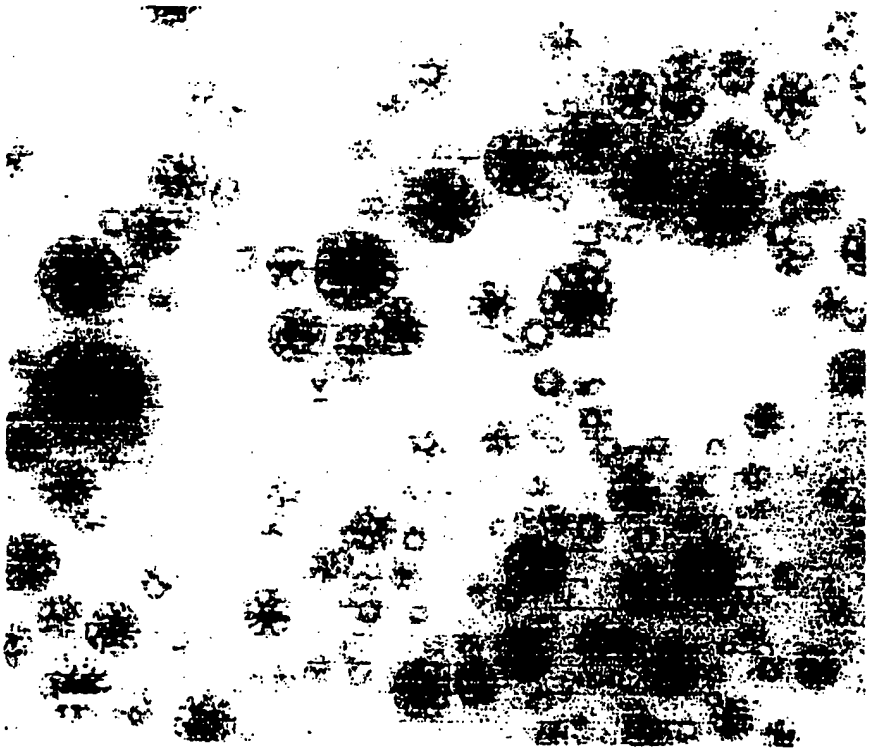


FIG 5B.

Response of A/J mice to Membrane Antigen Extract-ISCOMs

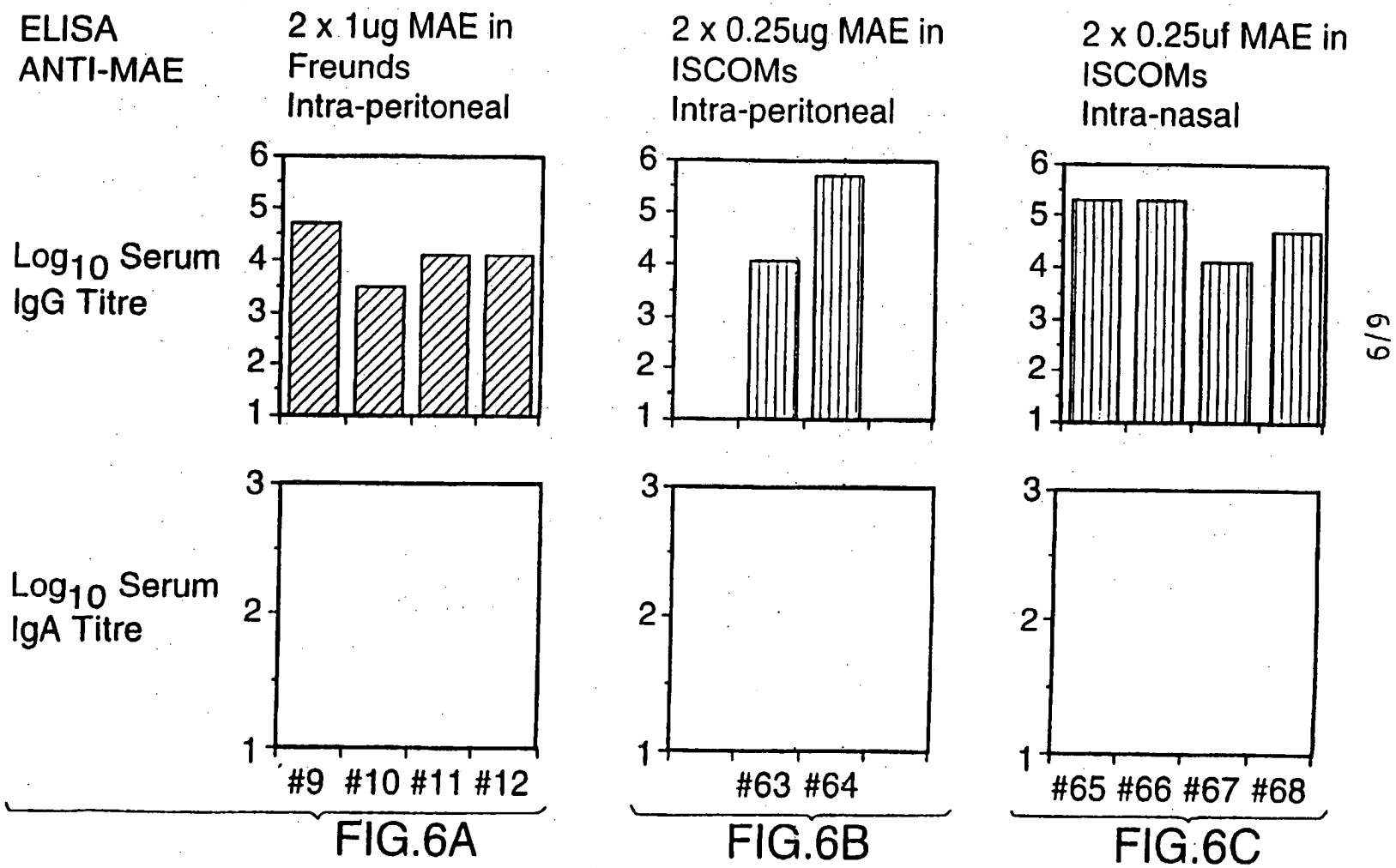


FIG.6A FIG.6B FIG.6C

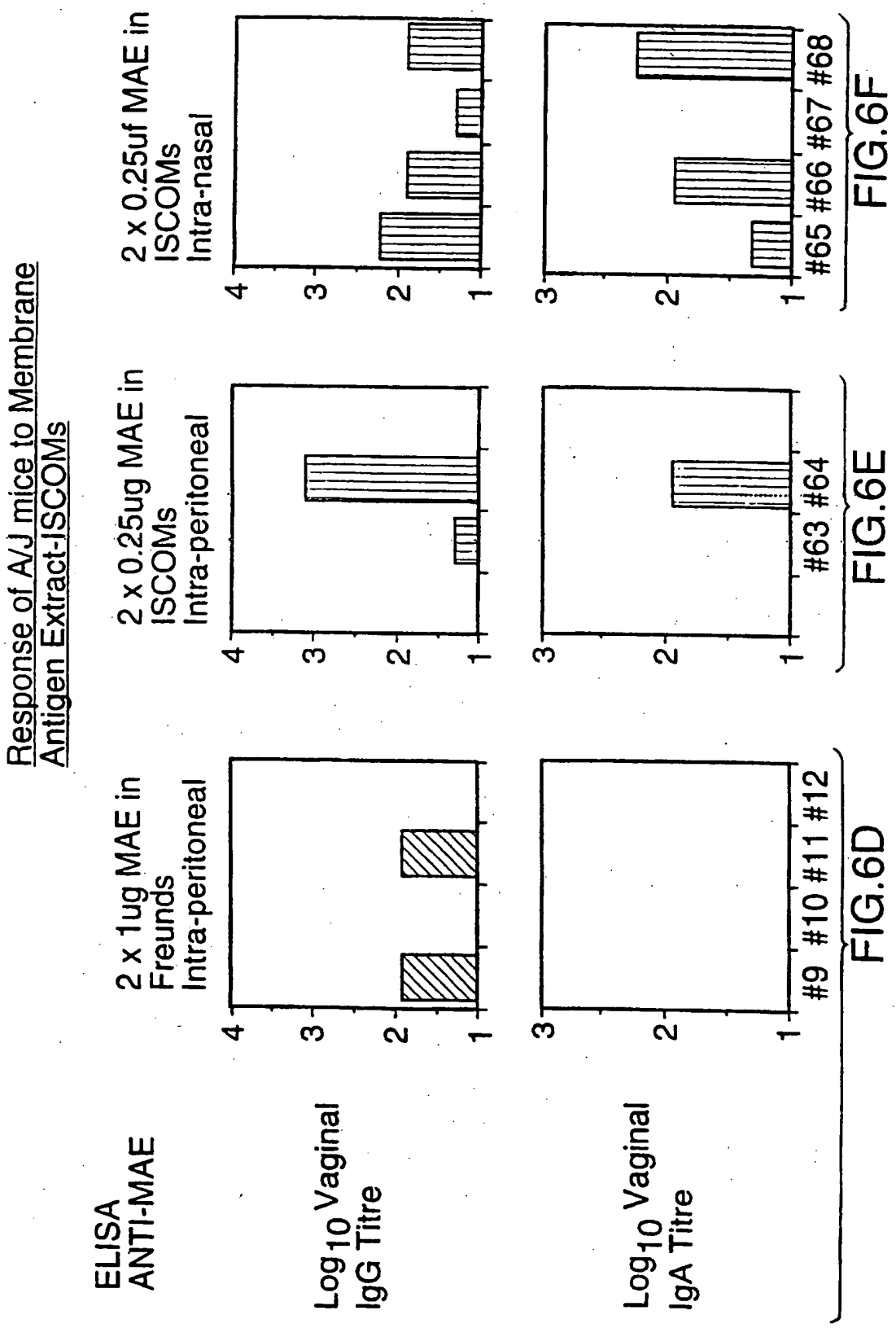
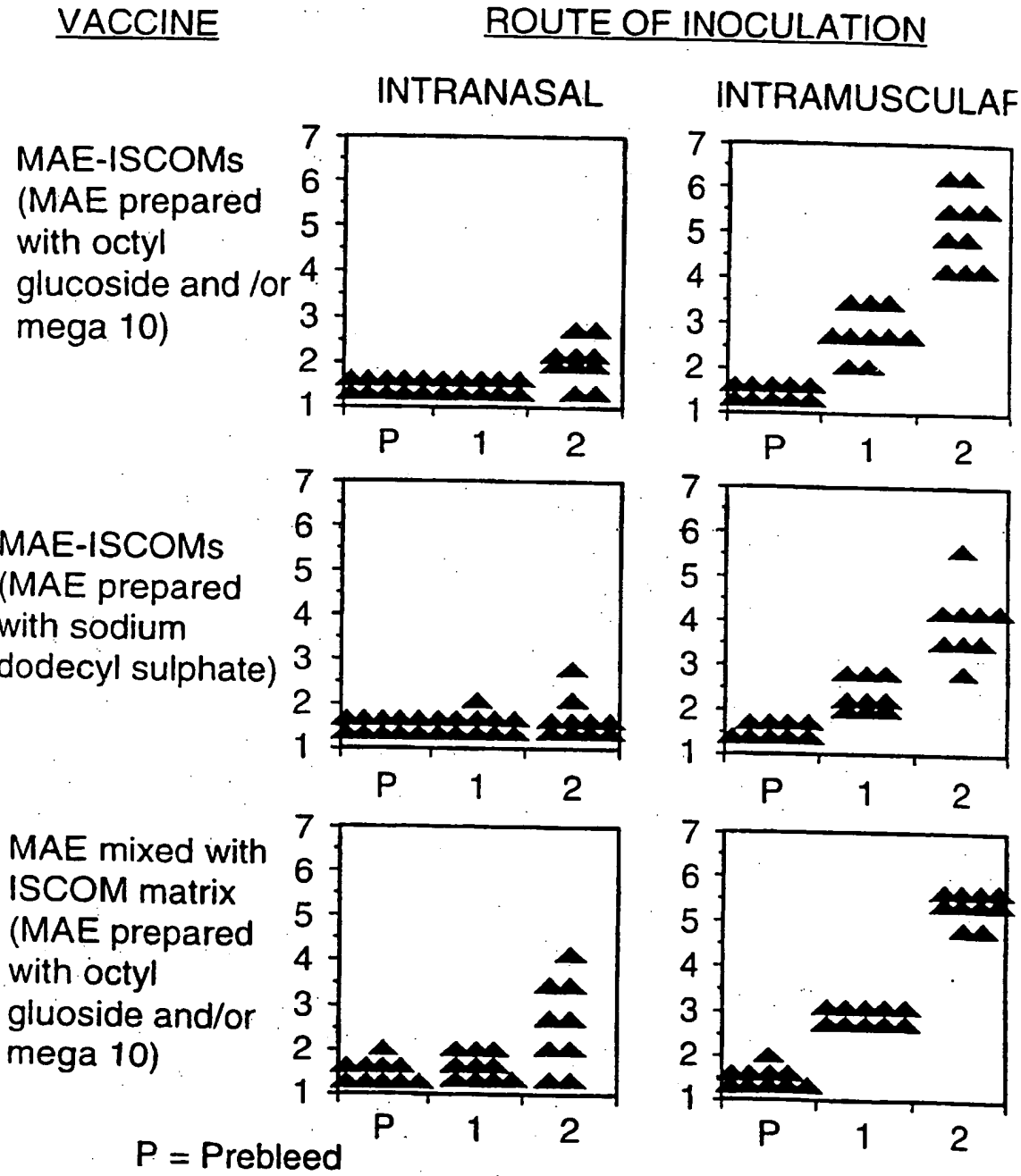


FIG.6D FIG.6E FIG.6F

RESPONSE OF C3H MICE TO MAE-ISCOMS
LOG_{10} SERUM IgG ANTI-MOMP ELISA TITRE

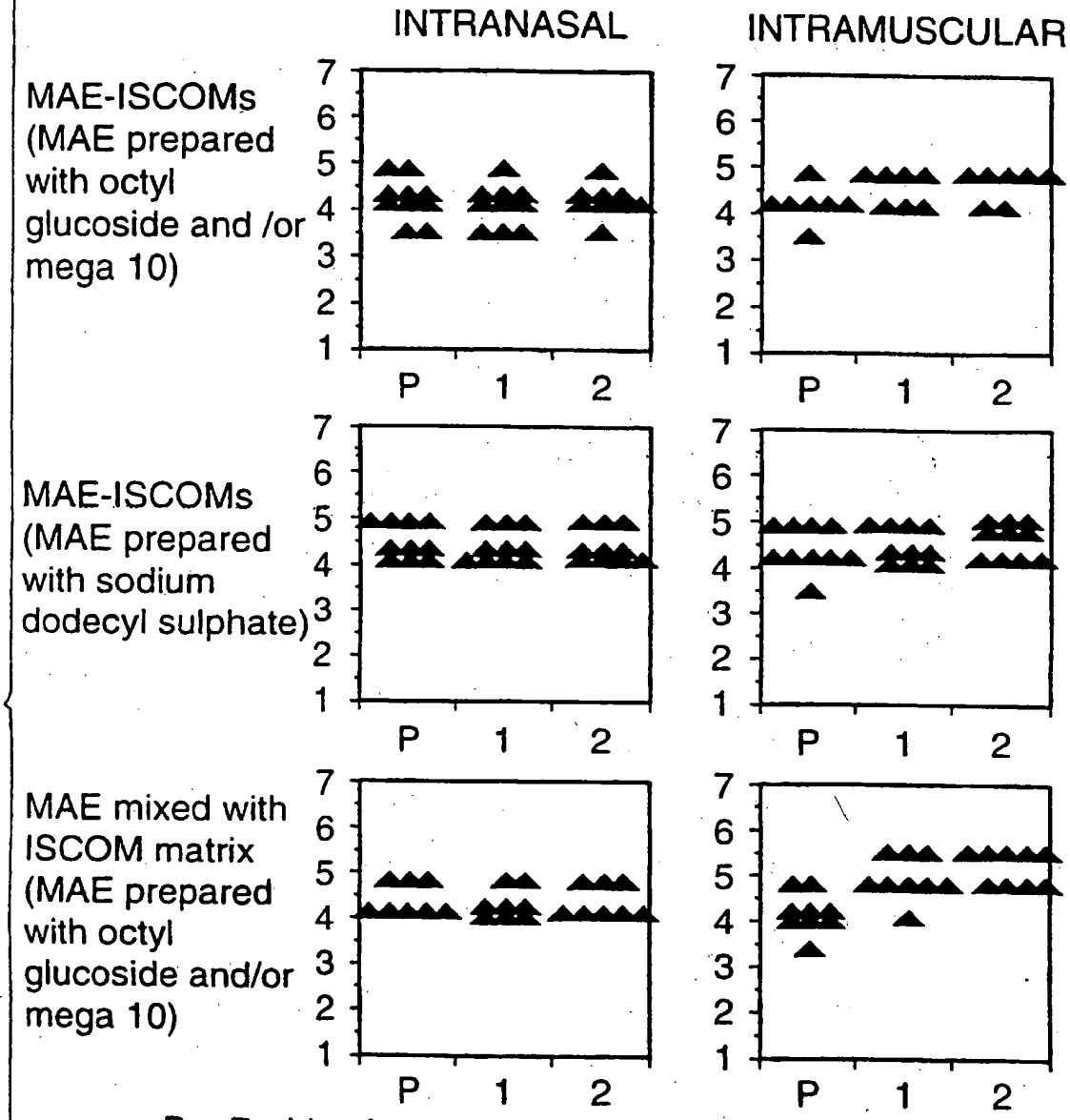


P = Prebleed
1 = 21 days after first inoculation (2ug MOMP)
2 = 21 days after second inoculation (2ug MOMP)
Each point represents the titre of a single mouse.

FIG.7

RESPONSE OFPREVIOUSLY INFECTED C3H MICE TO MAE-ISCOMS

LOG_{10} SERUM IgG ANTI-MOMP ELISA TITRE



P = Prebleed
1 = 21 days after first inoculation (2ug MOMP)
2 = 21 days after second inoculation (2ug MOMP)
Each point represents the titre of a single mouse.

FIG.8

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00656

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/118 C07K14/295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JONES G E ET AL: "Efficacy trials with tissue-culture grown, inactivated vaccines against chlamydial abortion in sheep." VACCINE, vol. 13, no. 8, 1995, pages 715-23, XP004057519 see page 715; abstract; last sentence see page 716; paragraph entitled "Vaccines" ---	1-9,21
Y	EP 0 415 794 A (MAWDSLEY MICHAEL JOHN) 6 March 1991 see page 10; example 10 see claim 1 --- -/--	1-9,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 1997	16. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mennessier, T

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00656

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SANDBULTE J ET AL: "Evaluation of Chlamydia psittaci subfraction and subunit preparations for their protective capacities." VETERINARY MICROBIOLOGY, vol. 48, no. 3-4, February 1996, pages 269-82, XP002050284 see the whole document	10-20, 22,23
Y	CAMPOS M ET AL: "A Chlamydial Major Outer Membrane Protein Extract as a Trachoma Vaccine Candidate." INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE 36 (8). 1995. 1477-1491. ISSN: 0146-0404, XP002050285 see the whole document	10-20, 22,23
Y,P	PAL S ET AL: "Immunization with an acellular vaccine consisting of the outer membrane complex of Chlamydia trachomatis induces protection against a genital challenge." INFECTION AND IMMUNITY, vol. 65, no. 8, August 1997, pages 3361-9, XP002050286 see the whole document	10-20, 22,23
A	EP 0 059 624 A (UNIV CALIFORNIA) 8 September 1982 see the whole document	10-18

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 97/00656

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically.

3. [ ] Claims Nos
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows.

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/CA 97/00656

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 7-9, 23 and 24

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claims 7-9, 23 and 24 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 97/00656

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0415794 A	06-03-91	AU 637405 B	27-05-93
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		AU 549025 B	09-01-86
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		JP 8035967 A	06-02-96